STRUCTURAL HOMOLOGY BETWEEN A MAMMALIAN H1° SUBFRACTION AND AVIAN ERYTHROCYTE-SPECIFIC HISTONE H5

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1. Introduction

In 1969 Panyim and Chalkley described a lysine-rich histone, now known as H1°, whose presence seemed to correlate with a lack of DNA synthesis [1]. These and other data which have emerged since add weight to the idea that H1° is a repressor of DNA synthesis [2].

We have been studying H1° and have found it to be characteristically different from all the other H1 subfractions from the same source and that it can also be separated into subfractions itself [3]. Here we report that homologies exist between the amino acid sequences of one of the subfractions of bovine H1° and the avian erythrocyte-specific histone H5.

2. Experimental

H1 and H1° were prepared as in [3]: whole ox liver was extracted with $HClO_4$ (5%, w/v), and the extract made 18% (w/v) CCl_3COOH. The precipitate was dissolved in water, made 5% (w/v) $HClO_4$, and re-precipitated by addition of HCl (0.3 M) and acetone (3.5 vol.).

Preparations were fractionated and subfraction H1°a prepared in pure form by chromatography on BioRex 70 ion exchange resin as in [3].

Cleavage of H1°a with cyanogen bromide was achieved with an excess of cyanogen bromide in formic acid (70%, w/v) for 40 h. Fragments so-generated were purified by descending paper chromatography in butanol/acetic acid/water/pyridine (30:72:288:240, by vol.) for 65 h.

A Rank Hilger Chromospek amino acid analyser was used to analyse peptides after their hydrolysis in HCl (6 M), 110°C, 24 h. No corrections were made for hydrolytic losses. N-terminal amino acids were determined by the dansyl method [4].

Sequence analysis of 2 mg peptide was carried out on a Beckman 890C Protein Sequenator using a quadrol (0.1 M) programme, essentially as in [5]. PTH derivatives of the released amino acids were determined both directly, by high-pressure liquid chromatography, and indirectly, by back hydrolysis to the free amino acid.

3. Results and discussion

Cleavage of H1°a with cyanogen bromide generated two peptides. On descending paper chromatography the smaller peptide (aCNBr1) moved away from the origin as a single band. The larger peptide (aCNBr2) remained at the origin. Total amino acid analyses of these peptides are given in table 1. That of aCNBr1 (together with its chromatographic mobility) indicates that it is ~30 residues long. The observation that aCNBr1 contains homoserine (produced from methionine during cleavage) and that its N-terminus is blocked (as is that of the parent molecule) places the methionine residue at position 30 (approx.). The amino acid sequence of H1°a from that position onwards was obtained from peptide aCNBr2 and is given in fig.1 together with H1 and H5 sequences for comparison. Fig.1 shows that this region of H1°a is much more like H5 from distantly-related species than like H1 from the same species. The greater number of differences between H1°a and chicken H5 than between chicken and goose H5 may be reconciled by the greater evolutionary divergence and the conservative nature of some changes. The position of the given H1°a sequence within the whole molecule is

Table 1
Total and N-terminal amino acid analyses of H1°a and derived peptides aCNBr1 and aCNBr2 (mol %)

	H1°a	aCNBr1	aCNBr2
Asp. (A)	3.3	10.6	2.3
Thr.	6.7	11.3	5.2
Ser.	9.3	17.1	7.6
Glut. (A)	5.4	6.0	4.5
Pro.	7.8	10.0	8.8
Gly.	4.4	4.3	4.8
Ala.	14.2	16.2	15.5
Val.	5.7	1.2	6.5
Cys.	0.0	0.0	0.0
Meth.	0.4	0.0	0.0
Isoleu.	2.6	0.0	2.4
Leu.	2.3	0.0	2.4
Tyr.	1.3	1.9	0.6
Phe.	1.2	0.7	1.0
Lys.)	31.1	17.3	33.6
Hist.) B	1.0	3.4	0.9
Arg.)	3.1	0.0	3.7
Acidics (A)	8.7	16.6	6.8
Basics (B)	35.2	20.7	38.2
B/A	4.0	1.2	5.6
N-terminal group	Blocked	Blocked	Ileu.

Homoserine was present in peptide aCNBr1. Data for H1°a from [3].

also more like that of the corresponding H5 sequences than of the H1 sequence. It seems likely that further sequencing in this region of H1°a will reveal further homology with H5.

The sequences of H1 and H5 given in fig.1 exist in the N-terminal parts of the globular regions of the whole molecule. These regions extend for some 70 residues from approximate positions 40 in H1 and 30 in H5 [7,8]. As pointed out [8] the central, globular region of H1 has remained highly conserved during evolution, although flanking sequences have proved to be variable. The same seems to be true for H5 [7], although the globular region of H5 is different from that of H1 (as fig.1 illustrates). It is therefore of considerable interest to find that a mammalian H1° subfraction has a region with a structure like that of the globular region of avian H5. Since the analysis of whole H1°a differs from avian H5, it follows that there must exist greater difference in other parts of the molecule. To our knowledge there has been no previous report of H5-like proteins in any cell other than nucleated erythrocytes and reticulocytes from birds, fish, amphibians and reptiles. This homology is, however, only apparent from sequence data. The total amino acid analysis of H1° or its subfractions [1,3] is not particularly H5-like, for it lacks the high arginine content usually associated with this protein, from avian erythrocytes [9]. It should be remembered, however, that H5 from non-avian sources, such as fish, contain less arginine, e.g., 6.8 mol/100 mol Arg in perch H5 [10]. Also, a lysine-rich fraction, F1b, has been found in turtle nucleated erythrocytes (which lacked a more obvious H5) but not in turtle liver, and the total amino acid analysis of F1b [11] is generally similar to that of bovine H1°. Avian H5 and mammalian H1° possibly represent two extremes of one family of proteins which share similar primary structures in their central regions.

Since the globular regions of H1 and H5 have each been highly conserved, it seems probable that these regions are important for the function of these histones, and any difference that there is between the functions of H1 and H5 might be a reflection of differences in structure of their globular regions. From this follow two points:

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BOVINE  H1°a  :   met-ileu-val-ala-ala-ileu-gln-ala-glu-lys-asn-arg-ala-gly-thr- X - X -gln- X -ileu

                  32
CHICKEN H5    :  -met-ileu-ala-ala-ala-ileu-arg-ala-glu-lys-ser-arg-gly-gly-ser-ser-arg-gln-ser-ileu-gln-

                  32
GOOSE   H5    :  -met-ileu-ala-ala-ala-ileu-arg-ala-asp-lys-ser-arg-gly-gly-ser-ser-arg-gln-ser-ileu-gln-

                  45
BOVINE  H1    :  -leu-ileu-thr-lys-ala-val -ala-ala-ser-lys-glu-arg-ser-gly-val-ser-leu-ala-ala-leu -lys-
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Fig.1. Comparison of partial amino acid sequences from bovine H1°a, chicken and goose H5, and a bovine H1 subfraction. H1°a sequence from peptide aCNBr2, with position of Met inferred by cleavage with cyanogen bromide. Unidentified residues are designated X. Reference for other data: chicken H5 [6]; goose H5 [7]; bovine H1 [8]. Numbers over first residues of H5 and H1 sequences indicate their positions within their respective whole molecules.

(i) The functions of H1 and H1° proteins might be markedly different;
(ii) Functions which have been tentatively assigned to H5 may also be assigned to H1°, and vice versa.

With regard to the latter point, the positive correlation of the occurrence of H5 and the shutting down of transcription during maturation of nucleated erythrocytes has prompted the suggestion that H5 is a repressor of RNA synthesis, (e.g. [12], discussed in [13]). Correlation of H1° occurrence with levels of transcription is less good than its correlation with a lack of DNA synthesis (e.g. [1,2]) so it might be that the primary function of H5 is in fact to repress DNA synthesis, which phenomenon also occurs during erythrocyte maturation. Further study of H1° proteins is progressing in our laboratory.

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